

passivation chemical such as bovine serum albumin (BSA), PLURONICS® (a tri-block copolymer), acrylic acid (AA), acrylamide (AM), dimethylacrylamide (DMA), 2-hydroxyethylacrylate (HEA), or polyethylene glycol (PEG) monomethoxylacrylate. The passivation chemical may be introduced onto the region or regions of microfluidic platform **10** which are to be passivated (e.g., channel **18**) and permitted to remain for a sufficient period of time (e.g., at least one hour). Alternatively, known ultraviolet (UV) graft polymerization processes may be employed to passivate one or more regions of microfluidic platform **10**.

[0065] Turning now to **FIGS. 12 and 13**, an exemplary specific binding assay apparatus **50** with which microfluidic platform **10** (**FIGS. 1 and 2**) may be used is depicted. Specific binding assay apparatus **50** may comprise a waveguide (e.g., planar, cylindrical, etc.) or a substrate carrying an array of waveguides and may be useful in fluorescence type assays or SPR type assays. Alternatively, specific binding assay apparatus **50** may comprise a semiconductor-based assay apparatus to which capture molecules have been secured. Microfluidic platform **10** may also be used with any other type of specific binding assay apparatus that may be used to detect analytes in samples or sample solutions that have very small volumes (e.g., volumes on the order of about a nanoliter ( $10^{-9}$  L) or less).

[0066] As depicted, specific binding assay apparatus **50** includes a reaction surface **52** with a plurality of sensing zones **54** arranged thereon in discrete locations. Each sensing zone **54** includes capture molecules **56** (e.g., proteins, peptides, nucleotides, etc.) that have been directly or indirectly immobilized to reaction surface **52**, as known in the art. Different sensing zones **54** may include capture molecules **56** with different analyte-binding specificities, or a plurality of different sensing zones may include the capture molecules **56** with the same analyte-binding specificity. When a microfluidic platform **10** according to the present invention is used with specific binding assay apparatus **50**, detection of one or more analytes may be effected at each zone with a relatively small number of analyte molecules (e.g., 10,000 or less, 1,000 or less, etc.). Accordingly, each sensing zone **54** may include a correspondingly small number of immobilized capture molecules **56**. The number of capture molecules bound at each sensing zone of a specific binding assay apparatus may be optimized or minimized based on the flow characteristics of a sample or sample solution through the microfluidic channel.

[0067] Although specific binding assay apparatus **50** is depicted as including a 3x3 array of sensing zones **54**, a microfluidic platform **10** according to the present invention may be used with specific binding assay apparatus that include arrays of sensing zones **54** with different organizations (e.g., other than area arrays and arrays that are not square, such as random, pseudorandom, and hexagonal arrays), as well as smaller or much larger arrays (e.g., 30x30 and larger) of sensing zones **54**.

[0068] In use of microfluidic platform **10** with a specific binding assay apparatus **50**, a sample or sample solution is introduced into a channel **18** of microfluidic platform **10** (**FIGS. 1 and 2**) and permitted to flow therethrough, such as by capillary action or by application of a positive or negative pressure to channel **18**. As the sample or sample solution flows along the length of channel **18**, the constituents of the

sample or sample solution, including any analytes therein, come into contact with capture molecules **56** (**FIG. 13**) that have been immobilized relative to reaction surface **52** of specific binding assay apparatus at one or more sensing zones **54** thereof. As analyte molecules within the sample or sample solution come into contact with corresponding capture molecules **56** at one or more sensing zones **54**, capture molecules **56** bind, by affinity interaction, their corresponding analytes.

[0069] Detection of such binding may then be effected by known processes. For example, if a direct, sandwich-type assay is to be performed, a tagged molecule (e.g., an antibody that has been tagged, or labeled, with a fluorescent dye or metal particle) that will specifically bind to each captured analyte may be introduced into channel **18**, flow therealong, and be permitted to bind to the analyte. The tag may then be stimulated into excitation and the excitation detected and correlated with an amount of analyte present in the sample or sample solution or simply with the presence or absence of the analyte in the sample or sample solution. Alternatively, the use of polymers to detect binding, or hybridization, of an analyte with a capture molecule may be used. Such processes are described in Boissinot, M, et al., "Detection of Nucleic Acids Using Novel Polymers Able to Transduce Hybridization into Optical or Electrical Signal," Micro Total Analysis Systems Conference, 2001, pages 319-20, the disclosure of which is hereby incorporated in its entirety by this reference.

[0070] As another example, a competitive binding-type assay of a type known in the art may be performed. In a competitive binding assay, tagged molecules that compete with a particular analyte for binding sites on capture molecules **56** are added to the sample or sample solution before introduction thereof into channel **18**. Because these tagged molecules compete with corresponding analyte molecules to bind to corresponding capture molecules **56**, the amount of the tag detected at a sensing zone **54** is inversely proportional to the amount of analyte in the sample or sample solution. Competitive binding assays are useful for detecting the binding of analytes by corresponding capture molecules **56** in real-time, as such binding occurs, or close to real-time.

[0071] As another alternative, when SPR is used to detect the amount or presence or absence of analytes in a sample or sample solution, each sensing zone **54** of specific binding assay apparatus **50** may include a cluster of metallic nanoparticles to which capture molecules **56** are tethered. As a sample or sample solution flows along the length of microfluidic channel **18** (**FIGS. 1 and 2**) and is introduced to each sensing zone **54**, analyte, if any, within the sample or sample solution may specifically bind to capture molecules **56** in that sensing zone **54**. An optically-transduced signal, which has an intensity that corresponds to the number of bound analyte molecules, may then be detected, as known in the art. Binding may be detected in real-time or close to real-time, as binding of analyte molecules by capture molecules **56** occurs.

[0072] Although the foregoing description contains many specifics, these should not be construed as limiting the scope of the present invention, but merely as providing illustrations of some of the presently preferred embodiments. Similarly, other embodiments of the invention may be devised which do not depart from the spirit or scope of the